American Journal of Hematology 60:285–288 (1999)

Autologous Rosette Formation by Human Blood Monocyte-Derived Macrophages and Lymphocytes

Humberto R.A. Cabral* and Ivon T.C. Novak

Instituto de Biologia Celular, Cátedras I y II de Histología, Embriologia y Genética, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina

The formation of rosettes between human blood monocyte-derived macrophages and lymphocytes (MLR) in samples harvested from total leukocyte (TL) cell cultures, was confirmed. Experiments with leukocytes obtained from human blood of healthy individuals (n = 17) and prepared under various conditions, were performed. Cytopreparations of each experiment were used for classical staining procedures or for immunohistochemical methods with monoclonal lymphocyte surface markers. Recently obtained blood leukocytes were unable to form MLR, whereas cultured samples of the same cells started to form MLR 15 hr after culturing. At that time, the number of MLR in pelleted samples was 1.18%, reaching a peak of 15.7% at 120 hr of culturing. In cultured but nonpelleted samples, only a few MLR were formed. With monoclonal antibodies, the lymphocytes forming MLR reacted mainly as CD4 positive and much less as CD8 (the ratio was 18:1). Monocyte-derived macrophages were able to form MLR when they underwent transformation into macrophages. The finding that the lymphocytes involved are T-cells, mainly CD4 positive, suggests that in the cell–cell interaction, macrophages could be presenting antigens to the lymphocytes. Besides, because the highest number of MLR occurred in TL samples, whereas few rosettes were formed in the mononuclear cell samples, the existence of some particular mechanism(s) acting on TL samples is suggested. Am. J. Hematol. 60:285–288, 1999. © 1999 Wiley-Liss, Inc.

Key words: autologous rosetting; cultured human macrophages; lymphocytes

INTRODUCTION

Blood monocyte-derived macrophages and lymphocytes, when harvested from cell cultures of total human leukocytes, showed the ability to form rosettes (MLR) between them, as we reported previously [1]. The other types of blood leukocytes, also present, did not form rosettes. The MLR are formed by a central macrophage surrounded by small lymphocytes [1].

Considering the selective cell–cell interaction in the MLR, as well as its occurrence in all the cases reported previously, we performed the present study with the aim of analyzing the formation of these rosettes under different experimental conditions.

MATERIAL AND METHODS

Human Blood Samples

Blood samples were obtained from 17 normal adult volunteers who gave their written informed consent (9 women, 8 men; mean age 27.2 years, range 18–61 years). Venous blood was withdrawn with sterile single-use syringes containing phenol-free heparin.

Study Design

Blood samples of each case were allowed to sedimentate by gravity for 2 hr. Plasma supernatants containing the total leukocytes were separated. At the same time, mononuclear cells were obtained from each blood sample by means of the Ficoll-Hypaque gradient, centrifugation method, according to Böyum [2].

Contract grant sponsor: SECYT, Córdoba, Argentina; Contract grant sponsor: CONICOR, Córdoba, Argentina.

*Correspondence to: Dr. Humberto R.A. Cabral, Instituto de Biología Celular, Cátedra I de Histología, Embriología y Genética, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina.

Received for publication 23 December 1997; Accepted 2 December 1998

TABLE I. Rosette Formation Between Human Monocyte-Derived Macrophages and Lymphocytes, Harvested in Samples of Autologous Total Leukocytes Cultures

Healthy subjects. Samples studied n = 17[a]	Time of cell culture						
	15 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr
Rosettes formed in cell culture samples pelleted at 200 *g*	1.18 ± 0.8	2.68 ± 1.29	4.63 ± 1.21	7.58 ± 2.18	10.43 ± 3.38	15.7 ± 5.04	14.5 ± 4.9
Rosettes formed in nonpelleted cell culture samples	0.1 ± 0.32	0.25 ± 0.44	0.41 ± 0.7	0.52 ± 0.7	0.82 ± 0.88	1.17 ± 0.80	1.02 ± 0.52

[a]Results are expressed as the mean of % number of macrophages that form rosettes, considering 100 of such cells in the respective cytopreparations.

The samples of total leukocytes (TL), and of mononuclear cells (MC) were used for the following experiments, respectively:

1. Freshly prepared TL or MC were smeared onto glass slides.
2. Other aliquots of freshly prepared TL or MC were centrifuged at 200*g* and cytopreparations were made with the respective cell pellets as follows: the cell pellets were gently resuspended in drops of TC199 culture medium and distributed with Pasteur pipettes onto identified glass slides and left to settle for 5 min, then desiccated by speedy rotation over a disk fixed on the axis of a centrifuge [3].
3. Other aliquots of the TL or the MC, respectively, were cultured at 37°C in TC199 medium supplemented with penicillin-streptomycin and 25% filtered autologous serum. Cultured cells were harvested at various times—15, 24, 48, 72, 96, 120, and 144 hr—with previous shaking of the culture flasks, and cytopreparations were made from each one, in a similar way to that described for 1 and 2, respectively.
4. Cytopreparations from each experiment were stained with Hematoxylin-Eosin or with May Grünwald Giemsa, whereas other cytopreparations were used for immunohistochemical methods with monoclonal antibodies for Human T-Cell UCHL-1, MT2 (CD45R), CD4 (T4), CD8, anti B-Cell (MB2), and Biotin-streptavidin, Alkaline Phosphatase method (supplied by BioGenex, San Ramon, CA).

The study of the cytopreparations was made by light microscopy. A macrophage-lymphocytes rosette was considered to be formed when a macrophage was surrounded by three or more lymphocytes. The characterization of the cells was defined by the results of the classic and the immunohistochemical techniques. The number of rosettes was expressed as the percentage of the number of macrophages that formed rosettes in a given cytopreparation by counting 100 macrophages.

RESULTS

In all the cases studied, the formation of rosettes between blood monocyte-derived macrophages and lymphocytes in ''in vitro'' cultures, was confirmed. MLR did not occur when the cells were just freshly obtained from blood—either in the directly prepared smears or in the cytopreparations performed with noncultured centrifuged cell pellets. MLR were observed in the cytopreparations from cell cultures; about 15 hr from the beginning of cultures, the monocytes started to show morphologic signs of their transformation into macrophages.

A low number of MLR was formed in the cytopreparations from cultured samples that were not centrifuged. Conversely, when the cultured cells were centrifuged and pelleted, a higher number of MLR was formed on the cytopreparations, as compared with noncentrifuged ones (Table I, Fig. 1).

The number of MLR formed in the pelleted cytopreparations from the total white cell cultures was higher than the number of MLR formed in the case of pellets of cultures of mononuclear cells obtained by Böyum's method [2] (Table II).

In cultured cells, an increment in the number of MLR formed was found in all cases with time of culturing, which reached a peak at 120 hr (Tables I and II).

In the case of TL, the pellets contained all the types of white blood cells and platelets, whereas in the case of MC, they contained lymphocytes, monocyte-macrophages and few platelets, being the presence of other leukocytes of less than 1%.

After immunostaining with monoclonal antibodies to recognize human lymphocyte cell surface markers, it was found that the lymphocytes forming the MLR were characterized as positive for UCHL-1. They did not react either with MT2 or with MB2 antibodies. They were also positive for CD4, and some lymphocytes reacted to CD8. In these MLR, the ratio between CD4+ and CD8+ was 18:1. Overall, these results indicate that the lymphocytes forming these rosettes are activated T-cells, mainly reacting with CD4 receptors (Fig. 2, Table III).

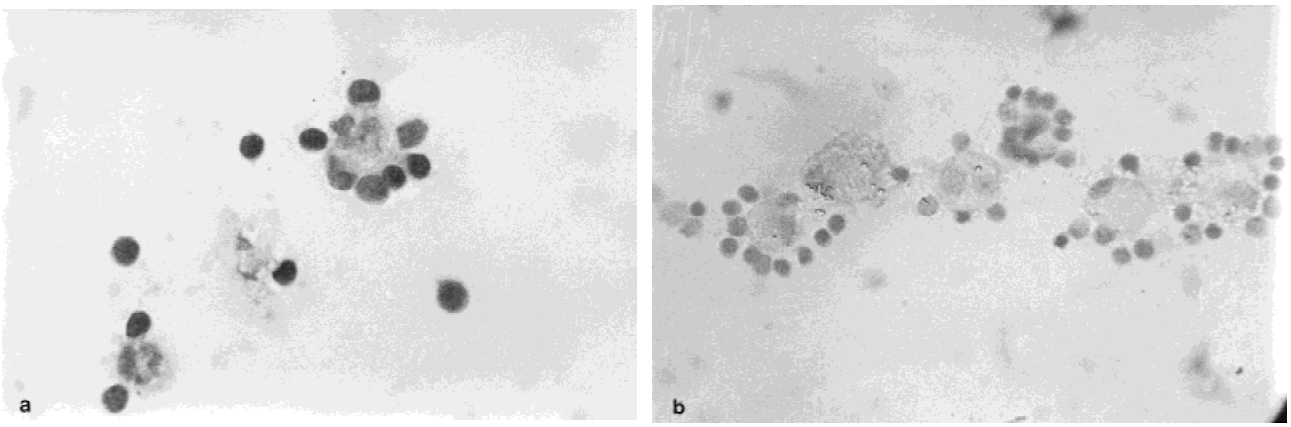

Fig. 1. a: Rosette formed between a human monocyte-derived macrophage and lymphocytes from a 48 hr total leukocyte culture—from a healthy subject. May Grümwald Giemsa, ×400. b: Rosettes formed between monocytge-derived macrophages and lymphocytes from a 120 hr total leukocyte culture—from a healthy subject. Hematoxylin Eosin, ×400.

TABLE II. Rosette Formation By Blood Mononuclear Cells Separated Using Ficoll-Hypaque Gradient and Cultured*

Samples studied n = 17	Time of cell culture						
	15 hr	24 hr	48 hr	72 hr	96 hr	120 hr	144 hr
Rosettes formed in MC cell culture samples pelleted at 200 *g*	0.0	0.0	0.7 ± 0.65	1 ± 0.80	1.4 ± 0.7	1.30 ± 0.9	1 ± 0.45
Rosettes formed in nonpelleted cell MC culture samples	0.0	0.0	0.0	0.1 ± 0.28	0.27 ± 0.4	0.4 ± 0.6	0.4 ± 0.65

*MC, mononuclear cells.

DISCUSSION

Current results on the formation of MLR only when monocytes undergo transformation into macrophages, and their occurrence in the samples obtained directly from the culture flask, indicate that this is not a laboratory artifact, and that these data could be of biological significance.

The finding that more rosettes were formed when cell culture samples were pelleted, suggests an enhancing action of this procedure, due to high cell density. Notwithstanding, this would be a phenomenon that takes place spontaneously, at a low rate, because some rosettes were found in the samples prepared directly from the cultures (without centrifugation), as shown in Table I. Besides, the formation of MLR into the culture flasks might indicate that the cell motility of both cell types involved contributes to the cell–cell interaction.

Other authors have described the formation of autologous cell rosettes with cells obtained from the spleen and lymph nodes of mice. (Inaba and Steiman [4], Breel, et al. [5], and Cumberbatch, et al. [6]). In those works, the rosettes were formed between antigen-presenting cells and lymphocytes and in all cases, the procedures used included a centrifugation step in order to obtain cell pellets.

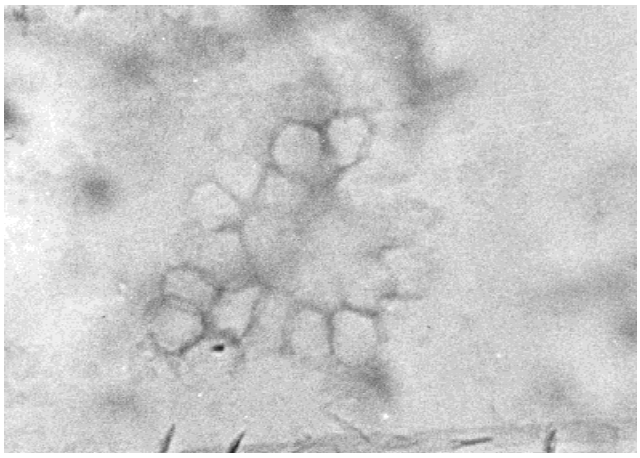

Fig. 2. Photomicrograph of an MLR stained with CD4 monoclonal antibody to T4 lymphocytes, using Alkaline Phosphatase label and Fast Red Chromogen. Note the positivity at the cell membrane of the lymphocytes. Citopreparation without nuclear counterstain.

In the present work, the procedure used to obtain the cell pellets was similar to the technique described by

TABLE III. Number of Lymphocytes That React With Different Monoclonal Antibodies in Macrophage-Lymphocytes Rosettes, Counted in 50 MLR for Each Case*

Monoclonal antibodies	Number of marked lymphocytes[a]
UHCL1	293 ± 14.05
CD4	289 ± 12.5
CD8	16 ± 4.37
MT2	0
MB2	0
Haematoxylin only	297 ± 13.35

*MLR, monocyte-derived macrophages and lymphocytes rosettes. Haematoxylin was used as counterstain. Cytopreparations were made at 120 hr of culture.

[a]Results are expressed as means ± SD, n = 10 for each experiment.

Jondal et al. [7] to form the E-rosettes between T-lymphocytes and sheep erythrocytes.

Besides, from our experiments, it appears that the transformation of blood monocytes into macrophages, is a necessary step to reach the ability to form MLR. Likewise, we found that the approximation between the cells facilitates, in some manner, MLR formation. Under these conditions, one of the roles that macrophages can perform, is the presentation of antigens to T-lymphocytes [8]. Insofar as in these MLR the surrounding lymphocytes were T-cells, a possible role in the antigen presentation step for this cell–cell interaction, cannot be ruled out.

The finding that the highest number of MLR occurred in TL samples, whereas only a few of them appeared in the MC samples, suggests the existence of some particular mechanism(s) in the first case. For instance, the presence of neutrophils or of the materials derived from them during culturing, could provide a microenvironment that enhances the formation of MLR.

In this connection, it is of interest that as early as after 5 hr of culturing total leukocytes, many neutrophils showed nuclear morphologic changes and formed small cell clusters, and that these were frequently associated to a monocyte-macrophage. At 24 hr, there are many neutrophils with signs of cariolisis, and some macrophages contain rests of polimorphonuclear cells (unpublished observations). Considering the natural (spontaneous) death of neutrophils that takes place into the cell cultures flasks containing TL [9], this could be a source of autoantigens for the cells involved in the phenomenon of MLR. Furthermore, considering the well known participation of cell adhesion molecules in certain cell–cell interactions, further work on the functional significance of MLR ought to take into consideration the above aspects.

ACKNOWLEDGMENTS

This study was supported in part by SECYT and CONICOR of Córdoba, Argentina. The authors thank the volunteer donors of the blood samples.

REFERENCES

1. Cabral HRA, Novak ITC. Spontaneous formation of rosettes by autologous human monocyte-macrophages and lymphocytes in cell cultures. Rev Fac Cien Méd Córdoba 1992;50(2):25–26.
2. Boyüm A. Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 1968;21(Suppl 97):51–59.
3. Cabral HRA. ''Los Linfocitos en la Enfermedad de Chagas.'' Córdoba, Argentina: Universidad Nacional de Córdoba, Argentina; 1984. p 58.
4. Inaba K, Steinman RM. Accessory cell-T lymphocyte interactions. Antigen-dependent and independent clustering. J Exp Med 1986; 163:247–261.
5. Breel M, Mebius R, Kraal G. The interaction of interdigitating cells and lymphocytes in vitro and in vivo. Immunology 1988;63:331–337.
6. Cumberbatch M, Illingworth Y, Kimber Y. Antigen-bearing dendritic cells in the draining lymph nodes of contact sensitised mice: cluster formation with lymphocytes. Immunology 1991;74:139–145.
7. Jondal M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cell. J Exp Med 1972;136:207.
8. Sperber K. Mononuclear phagocytic cells. In: Sigal LH, Yacov R, editors. Immunology and inflammation: basic mechanisms and clinical consequences. New York: McGraw-Hill, Inc.; 1994.
9. Tanaka Y, Epstein LB, Brecher G, Stohlman F Jr. Transformation of lymphocytes in cultures of human peripheral blood. Blood 1963; 22:614–649.